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No effect of transient BVDV infection on weight gain in beef calves in New Zealand

Masterthesis
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1 Zusammenfassung

Ziel dieser Arbeit war es festzustellen, ob eine transiente Infektion mit dem Bovinen Virusdiarrhoe Virus (BVDV) einen Einfluss auf die tägliche Gewichtszunahme von neuseeländischen Kälbern fleischbetonter Rassen hat. Dazu wurde 1174 Kälbern von 7 verschiedenen Betrieben auf der Nord- und Südinsel Neuseelands zweimal Blut genommen und mittels eines Antikörper-ELISA Tests ihr Serostatus bestimmt. Das Gewicht der Kälber wurde dreimal gemessen, das erste Mal im Alter von 2-7 Monaten, dann mit 4-8 Monaten und das letzte Mal dritten Mal mit 12-18 Monaten. Aus diesen Werten wurde ihre tägliche Gewichtszunahme berechnet. Eine durchgestandene transiente Infektion definierten wir als ein positives ELISA Ergebnis bei der zweiten Blutprobe. Beim Vergleich der täglichen Gewichtszunahme seropositiver Tiere mit derjenigen seronegativer Tiere konnten wir keinen statistisch signifikanten Unterschied feststellen. Wir schliessen daraus, dass eine transiente Infektion mit dem BVD Virus keinen deutlichen Einfluss auf die Gewichtszunahme von Fleischkälbern hat.

2 Abstract

AIM: To assess if a transient infection with bovine viral diarrhoea virus is affecting the daily weight gain of beef calves in New Zealand.
METHODS: 1174 beef calves of 7 farms on the North and South Island of New Zealand were weighed 3 times and tested twice for BVD induced antibodies (ab ELISA).
RESULTS: No significant association was detected between transient BVD infections and daily weight gains of beef calves in this study.
CONCLUSION: Transient infections with BVDV do not seem to have any remarkable impact on daily weight gains of beef calves.
KEY WORDS: BVD, beef calves, average daily weight gains, transient infection

3 Introduction

Bovine viral diarrhoea virus (BVDV) is a virus known worldwide, causing major economic losses in dairy and beef production.
An infection of a pregnant cow with the virus can either result in the resorption of the embryo, immunological tolerance of the foetus, abortion, stillbirth, malformation or in the birth of a healthy seropositive calf. Non-pregnant susceptible cattle that are infected with the BVDV undergo a period of transient viremia. They will either show clinical signs of a mild acute gastroenteritis with diarrhoea, fever, nasal discharge, cough and anorexia or not show any clinical signs. Subsequently, they will respond with a strong immune response and produce enough neutralizing antibodies to eliminate the virus and be protected from further infections with the same BVD strain. These animals are referred to as transiently infected animals. Immunologically tolerant calves however, called persistently infected (PI) calves, act as a virus reservoir for the rest of their lives, shedding it with all secretions and excretions, potentially infecting other cattle. PI animals might appear weaker, smaller and more susceptible to diseases such as respiratory diseases but often they do not show any clinical signs at all (Selbitz et al 2007; Anonymous 2011).
Economic costs caused by BVDV vary strongly depending on the pathogenicity of the virus strain as well as on the herd immunity and on the stage of breeding cycle when the infection occurs. Nevertheless it is estimated that per million calvings the annual loss is between USD 20Mio and 57Mio (Houe 1999). In New Zealand, economic losses caused by BVDV are estimated to 45Mio NZD per year or 87 NZD per cow and year for dairy industry alone. This amount arises from increased abortion and calving induction rates, increased time from calving to conception as well as decreased milk production (Heuer et al 2007). According to different calculations, the annual costs of BVD in an average sized (322 milking cows) New Zealand dairy herd amount to 11'334 NZD or 35.19 NZD per cow and year (Reichel et al 2008). As for the beef industry, a mean loss of about €58 per cow and year (abortion, conception failure, re-absorption) was estimated using an epidemiological outbreak model in Scotland (Gunn et al 2004). Furthermore, the majority of New Zealand adult cattle, around 60%, are thought to carry antibodies against BVDV (Littlejohns and Horner 1990) and up to 14.6% of the dairy herds are infected meaning that there are PI animals among them (Thobokwe et al 2004).
Due to considerable financial losses several European countries started to control and eradicate BVD. In Scandinavia, a two-stage eradication programme was implemented; in a first step, infected herds were identified by serological tests, in a second step all animals of an infected herd were tested and the virus-carrying animals were removed. Subsequently, non-infected herds were monitored by repeated sampling (Lindberg and Alenius, 1999). Currently, all Scandinavian countries are either free or almost free from BVD (Ståhl and Alenius, 2012).
In Switzerland, an eradication program was carried out from 2008-2012. Firstly, all bovines that went to summer pasture were sampled and tested for the virus before leaving. Within one year, the whole cattle population of Switzerland was sampled and tested for the virus. In the next phase (2009-2012), ear notches of every new born calf were taken and tested. Since 2013, the BVD free status of the herds is monitored by bulk tank milk or blood samples (non-lactating bovines) (Presi 2010). In New Zealand, control of BVD is based on voluntary, individual testing and eradication of PI animals as well as vaccinating against the disease. Efforts to eradicate New Zealand from BVD are in progress but to the authors knowledge no nationwide eradication programme has been started yet.
Whereas the effect of persistent infection with BVDV on animal performance has been studied intensively, information regarding the effect of transient infection on the average daily weight gains of beef calves is scarce. The aim of this study was therefore to assess if a transient BVDV infection is significantly associated with average daily weight gain in beef calves in New Zealand.

4 Material and Methods

Study animals

The animals in this study were originally targeted and sampled for a study on production effects of leptospirosis infection in calves, heifers, lambs and deer: "Effects of leptospirosis on pastoral livestock production in New Zealand" (provisional title, Emilie Vallee, unpublished data). The farms were chosen due to their size and proved presence of leptospirosis. Overall 1174 beef calves (Angus, Salers, Hereford, South Devon, Shorthorn and Crossbred animals) on 7 farms in 4 different districts (2 in Wairoa, 2 in Manawatu, 2 in Hurunui, 1 in Tararua district) on North and South Island of New Zealand were enrolled in this study. Farms were named E1, E2, E3, E4, E6, E7, E8. The calves were chosen randomly, not all calves of each farm were included. They were raised as suckler calves and therefore fed on milk and grass pasture during the entire time of the study. All calves of farm E7 were vaccinated against BVD, the calves of the other farms did not receive a vaccination. However, some of the calves' dams (all heifers of farm E2 and E6) were vaccinated at mating. Exact weaning dates of the calves are unknown but in general weaning dates of 5 to 7 months of age with a mean of 6 months can be assumed.

Study design and sample collection

Blood samples of all calves were collected twice and weights measured three times starting in November 2011 and continuing through April 2013. The blood samples were tested for BVDV induced antibodies (ab ELISA). Average daily gains were calculated for each animal. Finally, average daily weight gains of seropositive calves were compared to those of seronegative calves.
The study was designed as a cross-sectional study. Based on the ELISA result the animals were either classified as seropositive, seronegative or equivocal.
The calves were between 2 and 7 months of age when the first blood samples and first weights were recorded. Last blood samples and weights were collected when the calves were between 12 and 18 months of age. They were collected on the same day except on farm E1 where the last weights were measured approximately 4 months after the second blood samples were taken.
Weights were measured with the farmers own scales before releasing the animals back to their herd. Average daily gains were calculated for the period between first and third weight sampling (Adg1) and between second and third weight sampling (Adg2).
Blood samples were collected at the caudal vein using Red Top Vacutainers® and 20Gx1'' Vacutainer needles. The visual ID (ear tag) of each animal was written on its blood collection tube. The blood was allowed to clot and processed within 24h for serum collection. It was then stored at -20°C.

Serologic testing

The serum samples were tested by NZVP laboratories (New Zealand Veterinary Pathology Ltd) at Massey University in Palmerston North. An IDEXX BVDV p80 Ab Test® was carried out to detect BVD specific antibodies. The cut-offs for percentage inhibition were defined as recommended by the manufacturer as follows:
≥50 percentage inhibition (%INH) was considered as a negative result, 40-50% INH as doubtful/equivocal and <40% INH as a positive result.
A positive ELISA at the time of the second sampling was considered as indicative for a transient BVDV infection.

If equivocal results were added to positive results, it will be referred to as Spos, if they were added to negative results, it will be referred to as Sneg. The distinction was made for a sensitivity analysis of the results.

Statistical analysis

Statistical analysis was performed with the software R version 3.1.0 and the package nlme (Pinheiro et al. 2014).
With the aim to assess a potential association between serostatus and average daily weight gain, linear mixed effects models were used. The response variables were: Adg1, Adg2. The prediction variables were serostatus (Spos, Sneg or %Inhibition) and sex (F, M, MD (male - docked)), as fixed effects and farm as random effect. Model selection (inclusion of serostatus yes or no) was based on AIC (Akaike information criterion) and a likelihood ratio test of nested models was used to evaluate whether fixed or random effects significantly improved the model fit. Residuals were visually inspected for homoscedasticity, normal distribution of residuals and influential observations.

5 Results

The final analysis included 738 calves from 1174 initially enrolled calves with complete data on Adg1 and Adg2, sex, farm and serostatus. The remaining 436 animals were excluded mostly due to the last weight and/or blood sample being missing from farm E2.
The raw data (number of animals, number of females, males and docked males, serostatus, average daily weight gains as well as mean and standard deviation per farm) are summarized in Table 1.
On farm E1, nearly all calves were female. Two farms, E4 and E8, neutered their male calves whereas E3, E6 and E7 left the male calves intact. The calves of E1 as well as E3 had similar proportions of seropositive and seronegative calves at last sampling. However, the calves of E6, E7 and E8 were mainly seronegative, whereas on farm E4, nearly all animals were seropositive, hence within farm comparisons in these groups had low statistical power or were not possible at all. The overall average daily weight gain (Adg1) was around 600g for farms E1, E3, E4 and E6. Farm E7 with a mean of nearly 900g was considerably higher and E8 with 519g somewhat lower than the other farms.
Although the serostatus at the time of the second sampling was found to be significantly associated with average daily weight gains 1 and 2 (Figure 1), this association disappeared if farm was included in the model (either as fixed or as random effect, Figure 2). Similarly, no significant association was found between percentage inhibition and average daily weight gains if the effect of farm was included. Since one farm (E7) showed higher average daily gains (Figure 1) and had mostly negative animals, an analysis with a subset (only farm E1 and E3 which had both seronegative and seropositive animals) was performed to separate farm and a potential serostatus effect on weight. Again, serostatus was not significantly associated with average daily weight gains (Figure 3). The p-values are shown in Table 2.

6 Discussion

The aim of this study was to assess whether there was a significant change in average daily weight gain in beef calves that were suspected to have had a transient infection with BVDV prior to weighing, compared to beef calves without any signs of a past infection. Calves from 7 beef calf herds throughout New Zealand have been weighed and tested for BVDV induced antibodies. Daily body weight gains

of seropositive and seronegative calves were compared statistically. This study did not detect significant difference in average daily weight gains between calves with or without signs of a BVDV infection. However, the study suffered of low power since about 20% enrolled animals had to be excluded due to missing data. Moreover, only two farms had similar number of calves in the two exposure groups, hence allowed valid intra-farm comparisons. This reduced the statistical power further.

Farm effect

It appeared to be difficult to separate the effect of farm and serostatus. Therefore an analysis with a subset consisting of farms E1 and E3 was performed. Again, no association was evident between serostatus and daily weight gains. However, a marked effect of the sex was discovered. Male calves showed significantly higher weight gains than female calves.

Selection bias

A possible selection bias cannot be ruled out due to the following reasons: The animals were originally chosen for a study on leptospirosis and divided into two groups, of which one group received a vaccine against leptospirosis and the other one served as control group. Furthermore we lacked some information like BVD vaccination status of the mothers of the calves, biosecurity status of the farms, imported animals, calves that were not participating in the study but grazing on the same paddocks as the ones participating as well as contact to other herds over the fence. Lastly, we started with including 1174 calves in our study, but in the end only serum samples and corresponding weights of 738 calves could be obtained. Unfortunately many calves were not present at the last sampling (died, sold, unknown reasons) and their data was therefore lost. Some of these losses might have been caused by infection with BVDV. This loss could have biased the association between serostatus towards the null hypothesis of no Adg-difference (‚healthy worker effect'). Furthermore, no third weight was available from farm E2, hence farm E2 did not add any data to the study.

Transient infection

Transient infection was defined by an ab ELISA positive test result. However, sensitivity and specificity do not reach 100% and the results were not corrected for this. A positive test result does not have to be a transient infection, e.g. farm E7 with only five positive animals and a history of vaccinating all calves where positive titres could have been caused by vaccination. Moreover, there was uncertainty about the time of infection since only one sampling was taken into consideration on this farm. It is possible that some calves only got the infection a few weeks before the sampling and that there was therefore not enough time for developing an effect on weight gain.

Maternally derived antibodies

Two consecutive blood samples were tested for BVDV induced antibodies. However, only the second sample was statistically analysed because antibodies of the first sample might have been the tail end of waning maternal antibodies. Calves were between 2 and 7 months of age at the first sampling. Colostral BVDV antibody decay may range from 0 to200 days of age. The average half-life for BVD antibodies was found to be 20 days. (Menanteauhorta et al 1985). Another study about the decay of maternally derived BVD antibodies determined that after 114-141 days of age (depending on the type of BVD) half

of the calves in the study became seronegative. They suggested that the rate of decay is strongly associated with the initial antibody titre at day 1 to 3 of age (Munoz-Zanzi et al 2002). In view of such a large difference between studies, it is possible that some calves that were seropositive at the first sampling were carrying maternally derived antibodies.

Vaccine derived antibodies

All calves of farm E7 received a BVD vaccine as part of the farms management scheme. However, an influence of vaccine derived antibodies on the ELISA result seems unlikely as a study demonstrated that the commercially available inactivated BVD vaccine Bovilis® (Intervet International, the Netherlands) shows properties of a marker vaccine: after vaccinating cattle with a single or double shot, NS3 specific antibodies were low or undetectable, whereas after vaccination and subsequent challenge with BVD field virus all animals produced antibodies against NS3 and were positive in 4 different commercial ELISA tests. Vaccination with Bovilis® BVD does therefore not interfere with the development of antibodies after infection with field virus (Makoschey et al 2007).

Previous studies

Previous research about influence on weight gain associated with transient infection showed varying results: One study suggested no significant difference ($p > 0.05$) in average daily gain between calves in a pen (feedlots) containing PI animals and calves in a pen without PI animals in the herd. However, all calves in this study received a modified-live BVDV vaccine upon arrival at the feedlots and a booster vaccine up to 10 weeks later. No statistically detectable difference in morbidity, overall mortality or dry matter intake to gain ratio was found (Booker et al. 2008). In a different study, short (60h) or long term (entire finishing period 215d) exposure of 288 heifers directly or via adjacent pens to a PI animal did not show any significant differences in average daily gain, final body weight or overall efficiency of gain compared to non-exposed heifers. In this study as well the heifers received a vaccine against BVD before weaning and were given a metaphylactic antibiotic treatment upon arriving at the study site. It was suggested that exposing vaccinated heifers to a PI animal did not affect growth performance or mortality (Elam et al 2008). However, a tendency of reduced average daily gain (not statistically significant $p=0.09$) over the entire finishing period was shown in a study of 12 unvaccinated steers with short time (72h) exposure to PI animal. A long-term negative effect on animal performance after exposure was therefore suggested (Burciaga-Robles et al. 2010). Another study showed an exposure effect on average daily gains of calves having contact with a PI pen mate: the exposed calves showed smaller average daily gains from day 28 to day 42 of the study even though they were vaccinated against BVD. They also needed more antibiotic therapy and had more chronically ill animals in the same pen compared to calves that were not exposed (Richeson et al. 2012). Lastly, one study showed that animals with high antibody titre typically weighed less than animals without evidence of exposure. Other factors like stillbirth, abortion rate, calf-death and nonpregnancy however seemed to be the same as animals without evidence of exposure (Waldner and Kennedy 2008).

It can be concluded that according to our data there was insufficient evidence for an association between serostatus and daily weight gain of beef calves on these New Zealand farms.

7 Acknowledgements

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9 Tables and Figures

Farm	N[1]	Sex[2]		Spos[3]		Sneg[4]		Adg1[5]	Adg2[6]
		F	M	Pos	Neg	Pos	Neg	Mean (SD)	Mean (SD)
E1	72	68	3	11	61	11	61	626g (66)	640g (85)
E3	179	101	78	115	64	102	77	610g (86)	726g (186)
E4	170	90	80	167	3	167	3	639g (76)	592g (79)
E6	96	40	56	0	96	0	96	612g (72)	583g (106)
E7	184	87	92	6	178	5	179	894g (126)	850g (152)
E8	37	27	10	0	37	0	37	519g (84)	406g (70)

Table 1
1: Number of animals
2: Female, Male
3: Serostatus, equivocal results allocated to Positives
4: Serostatus, equivocal results allocated to Negatives
5: Average daily weight gains through entire sampling period, mean, standard deviation
6: Average daily weight gains through second half of sampling period

	Spos, (Sex)	Sneg, (Sex)	%INH, (Sex)
All farms, Adg1	0.822, (<.0001)	0.723, (<.0001)	0.816, (<.0001)
All farms, Adg2	0.559, (<.0001)	0.419, (<.0001)	0.563, (<.0001)
Subset E1+E3, Adg1	0.516, (0.040)	0.994, (0.0355)	0.985, (0.037)
Subset E1+E3, Adg2	0.917, (0.224)	0.901, (0.215)	0.933, (0.220)

Table 2: p-values

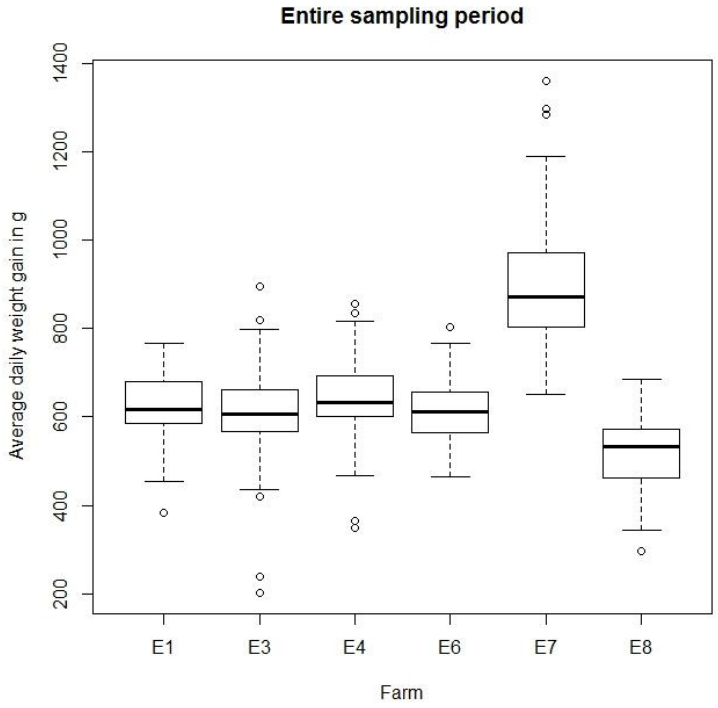

Figure 1
Average daily gains during the entire samplng period (November 2011- April 2013), which corresponds to Adg1.

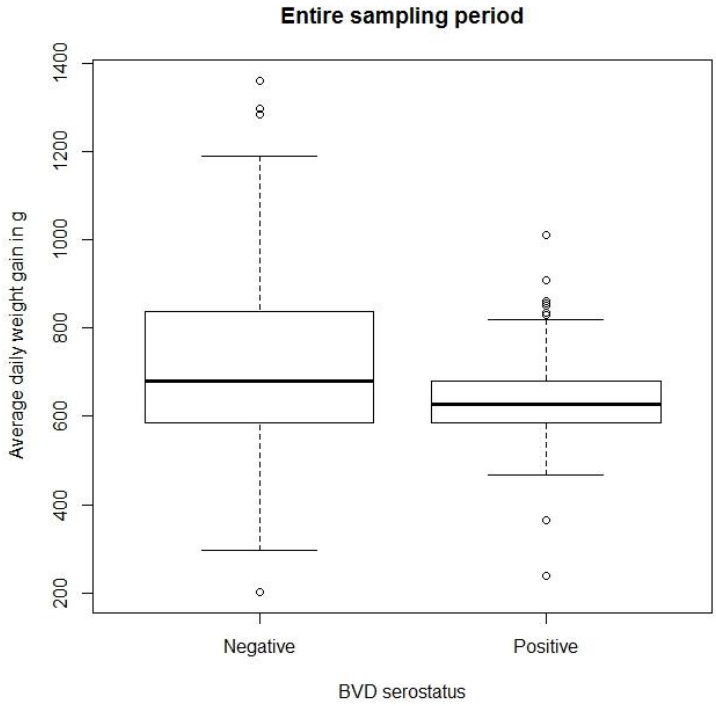

Figure 2
Comparing antibody positive and negative calves (all farms, Sneg) during the entire sampling period, which corresponds to Adg1.

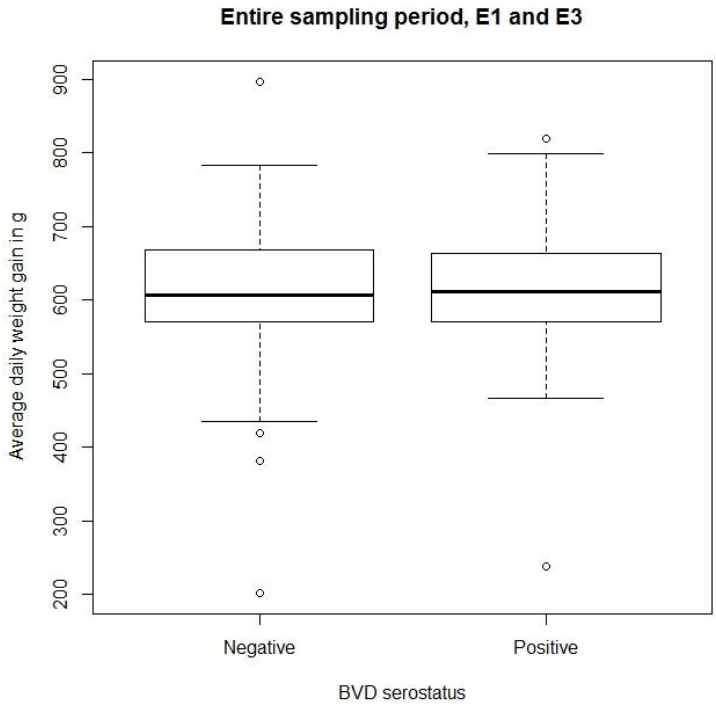

Figure 3
Comparing antibody positive and negative calves of farms E1 and E3 (subset, Sneg) during the entire sampling period, which corresponds to Adg1.

10 Eigenständigkeitserklärung

Ich erkläre hiermit, dass ich die vorliegende Arbeit ohne unlautere fremde Hilfe und ohne Verwendung anderer als der angegebenen Hilfsmittel verfasst habe, dass ich sämtliche verwendete Quellen erwähnt und gemäss gängigen wissenschaftlichen Zitierregeln nach bestem Wissen und Gewissen korrekt zitiert habe und dass ich alle Originaldaten vollständig und wahrheitsgetreu wiedergegeben habe.

Bubikon, 20.08.2014

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